

WHAT IS CLAIMED IS:

- 145  
1A1  
5
1. An isolated polynucleotide that:
- (a) encodes a polypeptide having PEAMT or  $\Delta$ PEAMT activity and that comprises an at least 27 contiguous amino acid sequence from SEQ ID NO:2 or SEQ ID NO:4;
- (b) encodes a polypeptide having PEAMT or  $\Delta$ PEAMT activity and at least about 85% sequence identity with the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4;
- (c) comprises an at least 26 contiguous nucleotide sequence from SEQ ID NO:1 or SEQ ID NO:3; or
- (d) hybridizes to the sequence of SEQ ID NO:1 or SEQ ID NO:3, or to the complement thereof, under stringent hybridization conditions.
2. The isolated polynucleotide of claim 1, comprising a sequence region that encodes a polypeptide having an at least 27 contiguous amino acid sequence from SEQ ID NO:2 or SEQ ID NO:4.
3. The isolated polynucleotide of claim 2, comprising a sequence region that encodes a polypeptide having an at least 29 contiguous amino acid sequence from SEQ ID NO:2 or SEQ ID NO:4.
4. The isolated polynucleotide of claim 3, comprising a sequence region that encodes a polypeptide having an at least 31 contiguous amino acid sequence from SEQ ID NO:2 or SEQ ID NO:4.

5. The isolated polynucleotide of claim 4, comprising a sequence region that encodes a polypeptide having an at least 33 contiguous amino acid sequence from SEQ ID NO:2 or SEQ ID NO:4.

- 5
6. The isolated polynucleotide of claim 5, comprising a sequence region that encodes a polypeptide having an at least 35 contiguous amino acid sequence from SEQ ID NO:2 or SEQ ID NO:4.

- 10
7. The isolated polynucleotide of claim 6, comprising a sequence region that encodes a polypeptide having an at least 37 contiguous amino acid sequence from SEQ ID NO:2 or SEQ ID NO:4.

- 15
8. The isolated polynucleotide of claim 7, comprising a sequence region that encodes a polypeptide having the sequence of SEQ ID NO:2 or SEQ ID NO:4.

- 20
9. The isolated polynucleotide of claim 1, comprising a sequence region that encodes a polypeptide having PEAMT or  $\Delta$ PEAMT activity and at least about 85% sequence identity with the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4.

- 25
10. The isolated polynucleotide of claim 9, comprising a sequence region that encodes a polypeptide having PEAMT or  $\Delta$ PEAMT activity and at least about 90% sequence identity with the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4.

- 30
11. The isolated polynucleotide of claim 10, comprising a sequence region that encodes a polypeptide having PEAMT or  $\Delta$ PEAMT activity and at least about 95% sequence identity with the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4.

12. The isolated polynucleotide of claim 11, comprising a sequence region that encodes a polypeptide having PEAMT or  $\Delta$ PEAMT activity and at least about 85% sequence identity with the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4.

13. The isolated polynucleotide of claim 12, comprising a sequence region that encodes a polypeptide having PEAMT or  $\Delta$ PEAMT activity and at least about 98% sequence identity with the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4.

14. The isolated polynucleotide of claim 1, comprising an at least 26 contiguous nucleotide sequence from SEQ ID NO:1 or SEQ ID NO:3.

15. The isolated polynucleotide of claim 14, comprising an at least 30 contiguous nucleotide sequence from SEQ ID NO:1 or SEQ ID NO:3.

16. The isolated polynucleotide of claim 15, comprising an at least 40 contiguous nucleotide sequence from SEQ ID NO:1 or SEQ ID NO:3.

17. The isolated polynucleotide of claim 16, comprising an at least 50 contiguous nucleotide sequence from SEQ ID NO:1 or SEQ ID NO:3.

18. The isolated polynucleotide of claim 17, comprising the nucleotide sequence of SEQ ID NO:1 or SEQ ID NO:3.

19. The isolated polynucleotide of claim 1, comprising a sequence region that hybridizes to the sequence of SEQ ID NO:1 from about position 254 to about position 1735, or to the sequence of SEQ ID NO:3, or to the complement of SEQ ID NO:1 or SEQ ID NO:3, under stringent hybridization conditions.

<sup>20</sup>  
21. The isolated polynucleotide of claim <sup>19</sup>20, comprising a sequence region that hybridizes under hybridization conditions comprising a salt concentration of from about 0.02 M to about 0.15 M, and a temperature of from about 50°C to about 70°C.

<sup>21</sup>  
22. An isolated polynucleotide that comprises:

- (a) a sequence region that consists of at least 26 contiguous nucleotides that have the same sequence as, or are complementary to, at least 26 contiguous nucleotides of SEQ ID NO:1 or SEQ ID NO:3; or
- (b) a sequence region of from 26 to about 10,000 nucleotides in length that hybridizes to the nucleic acid segment of SEQ ID NO:1 or SEQ ID NO:3; or the complement thereof, under hybridization conditions comprising a salt concentration of from about 0.02 M to about 0.15 M, and a temperature of from about 50°C to about 70°C.

<sup>22</sup>  
23. The isolated polynucleotide of claim <sup>21</sup>22, comprising a sequence region that consists of at least 26 contiguous nucleotides that have the same sequence as, or are complementary to, at least 26 contiguous nucleotides of SEQ ID NO:1 or SEQ ID NO:3.

<sup>23</sup>  
24. The isolated polynucleotide of claim <sup>22</sup>23, wherein said polynucleotide is from about 100 to about 10,000 nucleotides in length.

<sup>24</sup>  
25. The isolated polynucleotide of claim <sup>23</sup>24, wherein said nucleic acid segment is from about 500 to about 5,000 nucleotides in length.

<sup>25</sup>  
26. The isolated polynucleotide of claim <sup>24</sup>25, wherein said nucleic acid segment is from about 1000 to about 4,000 nucleotides in length.

26  
27.

5

The isolated polynucleotide of claim <sup>21</sup>22, comprising a sequence region of from 26 to about 10,000 nucleotides in length that hybridizes to the nucleic acid segment of SEQ ID NO:1 or SEQ ID NO:3; or that hybridizes to the complement thereof, under hybridization conditions comprising a salt concentration of from about 0.02 M to about 0.15 M, and a temperature of from about 50°C to about 70°C.

26  
28.

10

The isolated polynucleotide of claim <sup>26</sup>27, comprising a sequence region of from 30 to about 5000 nucleotides in length that hybridizes to the nucleic acid segment of SEQ ID NO:1 or SEQ ID NO:3; or that hybridizes to the complement thereof, under hybridization conditions comprising a salt concentration of from about 0.02 M to about 0.15 M, and a temperature of from about 50°C to about 70°C.

26  
29.

15

The isolated polynucleotide of claim <sup>27</sup>28, comprising a sequence region of from 40 to about 4000 nucleotides in length that hybridizes to the nucleic acid segment of SEQ ID NO:1 or SEQ ID NO:3; or that hybridizes to the complement thereof, under hybridization conditions comprising a salt concentration of from about 0.02 M to about 0.15 M, and a temperature of from about 50°C to about 70°C.

29  
30.

25

The isolated polynucleotide of claim <sup>28</sup>29, comprising a sequence region of from 50 to about 3000 nucleotides in length that hybridizes to the nucleic acid segment of SEQ ID NO:1 or SEQ ID NO:3; or that hybridizes to the complement thereof, under hybridization conditions comprising a salt concentration of from about 0.02 M to about 0.15 M, and a temperature of from about 50°C to about 70°C.

30

30  
31.

The isolated polynucleotide of claim <sup>29</sup>30, comprising a sequence region of from 60 to about 2000 nucleotides in length that hybridizes to the nucleic acid segment of SEQ ID NO:1 or SEQ ID NO:3; or that hybridizes to the complement thereof, under hybridization conditions

comprising a salt concentration of from about 0.02 M to about 0.15 M, and a temperature of from about 50°C to about 70°C.

5 <sup>31</sup>  
32. The isolated polynucleotide of claim <sup>30</sup>31, comprising a sequence region of from 70 to about 1000 nucleotides in length that hybridizes to the nucleic acid segment of SEQ ID NO:1 or SEQ ID NO:3; or that hybridizes to the complement thereof, under hybridization conditions comprising a salt concentration of from about 0.02 M to about 0.15 M, and a temperature of from about 50°C to about 70°C.

10 <sup>32</sup>  
33. The polynucleotide of claim 1 or claim <sup>21</sup>22, further defined as an RNA, a PNA, or a DNA segment.

15 <sup>33</sup>  
34. The polynucleotide of claim 1 or claim <sup>21</sup>22 comprised within a vector.

20 <sup>34</sup>  
35. The polynucleotide of claim <sup>33</sup>34, comprised within a plasmid, cosmid, phage, phagemid, baculovirus, virus, virion, bacterial artificial chromosome, or yeast artificial chromosome vector.

25 <sup>35</sup>  
36. The polynucleotide of claim <sup>34</sup>35, wherein said vector further comprises a promoter that is operably linked to said polynucleotide.

<sup>36</sup>  
37. The polynucleotide of claim <sup>35</sup>36, wherein said promoter is a heterologous promoter.

30 <sup>37</sup>  
38. The polynucleotide of claim <sup>36</sup>37, wherein said heterologous promoter is a plant-expressible constitutive, inducible, or tissue-specific promoter.

37  
239. The polynucleotide of claim 38, wherein said plant-expressible promoter is selected from the group consisting of corn sucrose synthetase 1, corn alcohol dehydrogenase 1, corn light harvesting complex, corn heat shock protein, pea small subunit RuBP carboxylase, Ti plasmid mannopine synthase, Ti plasmid nopaline synthase, petunia chalcone isomerase, bean glycine rich protein 1, Potato patatin, lectin, CaMV 35S, ALS, ubiquitin, globulin 1, cruciferin, napin,  $\beta$ -conglycinin, phaseolin,  $\gamma$  zein, and the S-E9 small subunit RuBP carboxylase promoter.

39  
40. A virus comprising the polynucleotide of claim 1 or claim 22.<sup>21</sup>

40  
41. A host cell comprising the polynucleotide of claim 1 or 22<sup>21</sup> or the virus of claim 40.

41  
42. The host cell of claim 41<sup>40</sup>, wherein said host cell is a bacterial cell.

42  
43. The host cell of claim 42<sup>41</sup>, wherein said host cell is an *Escherichia*, *Salmonella* or *Agrobacterium* cell.

43  
44. The host cell of claim 41<sup>40</sup>, wherein said cell is an eukaryotic cell.

44  
45. A composition comprising the polynucleotide of claim 1 or claim 22, the virus of claim 40, or the host cell of claim 41.<sup>40</sup>

45  
46. A nucleic acid detection kit comprising, in suitable container means, at least a first isolated nucleic acid segment comprising the polynucleotide of claim 1 or claim 22<sup>21</sup>, and instructions for using said kit.

46  
47.

A transgenic plant comprising:

(a) a heterologous nucleic acid segment that comprises the polynucleotide of claim 1;

5

(b) the virus of claim <sup>29</sup>40; or

(c) the host cell of claim <sup>40</sup>41.

10

47  
48.

The transgenic plant of claim 47, having stably incorporated into its genome a heterologous nucleic acid segment that comprises the polynucleotide of claim 1, wherein said polynucleotide is operably linked to a promoter that expresses said polynucleotide in said transgenic plant.

15

48  
49.

The transgenic plant of claim 48, wherein said plant expresses said heterologous nucleic acid segment to produce a polypeptide that has at least about 85% sequence identity with the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4.

20

49  
50.

The transgenic plant of claim 49, wherein said plant expresses said heterologous nucleic acid segment to produce a polypeptide that has at least about 95% sequence identity with the sequence of SEQ ID NO:2 or SEQ ID NO:4.

25

50  
51.

The transgenic plant of claim 50, wherein said plant expresses said heterologous nucleic acid segment to produce a polypeptide that has at least about 99% sequence identity with the sequence of SEQ ID NO:2 or SEQ ID NO:4.

30

51  
52.

The transgenic plant of claim 47, wherein said plant is a monocotyledonous or a dicotyledonous plant.



52  
53.

The transgenic plant of claim 52, wherein said plant is a grain, tree, legume, fiber, vegetable, fruit, berry, nut, citrus, grass, cactus, succulent, floral, or ornamental plant.

5 53  
54.

The transgenic plant of claim 53, wherein said plant is a corn, rice, millet, tobacco, alfalfa, soybean, bean, sorghum, pea, *Brassica*, safflower, potato, coconut, palm, pumpkin, squash, poppy, sesame, peanut, cocoa, coffee, tomato, flax, canola, sunflower, cotton, kapok, wheat, oat, barley, walnut, pecan, almond, or rye plant.

10 54  
55.

A progeny or seed of any generation of the transgenic plant of claim 47.

55  
56.

A progeny of any generation of the seed of claim 55.

15 56  
57.

A seed of any generation of the progeny of claim 56.

20 57  
58.

A plant grown from the seed of claim 57.

58  
59.

A method for detecting a PEAMT- or a  $\Delta$ PEAMT-encoding polynucleotide in a sample, comprising the steps of:

25

(c) contacting a population of polynucleotides suspected of encoding a PEAMT or a  $\Delta$ PEAMT polypeptide with at least a first labeled polynucleotide in accordance with claim 1 or claim 22, under conditions effective to allow hybridization of substantially complementary nucleic acids; and

30

(d) detecting the hybridized complementary nucleic acids so formed.

59 60.

A method of increasing the amount of PEAMT or  $\Delta$ PEAMT polypeptide in a plant cell comprising, expressing in said plant cell a biologically effective amount of the polynucleotide of claim 1.

5

60  
61.

A method of altering the level of phosphodimethylethanolamine, phosphomonomethylethanolamine, phosphatidylcholine, phosphocholine, choline, glycine betaine, or choline-*O*-sulfate in a plant cell comprising, expressing in said plant cell a biologically effective amount of the polynucleotide of claim 1.

10

61  
62.

A method of increasing the lipid content of a plant seed comprising, expressing in a transgenic plant an amount of the polynucleotide of claim 1 effective to increase the lipid content in the seed of said plant relative to the lipid content of a seed from an untransformed plant.

15

62  
63.

A method of increasing the choline or phosphatidylcholine content of a plant seed comprising, expressing in a transgenic plant an amount of the polynucleotide of claim 1 effective to increase the choline or phosphatidylcholine content in the seed of said plant relative to the choline or phosphatidylcholine content of a seed from an untransformed plant.

20

63  
64.

A method of decreasing the ethanolamine or phosphoethanolamine content of a plant seed comprising, expressing in a transgenic plant an amount of the polynucleotide of claim 1 effective to decrease the ethanolamine or phosphoethanolamine content in the seed of said plant relative to the ethanolamine or phosphoethanolamine content of a seed from an untransformed plant.

25

30

64  
65.

A method for altering the biosynthesis of a compound selected from the group consisting of phosphodimethylethanolamine, phosphomonomethylethanolamine, phosphatidylcholine, phosphocholine, choline, glycine betaine, and choline-*O*-sulfate in a plant, said

method comprising the steps of (a) transforming said plant with a selected nucleic acid segment that comprises the polynucleotide of claim 1 operably linked to a promoter that drives expression in said plant; and (b) growing the plant so transformed under conditions effective to modulate the biosynthesis of said compound in said plant.

65  
66.

The method of claim 65, wherein the biosynthesis of said compound in said transformed plant is elevated relative to that in an untransformed plant of the same species.

66  
67.

The method of claim 65, said method further comprising the steps of (c) growing said transformed plant under conditions effective for obtaining seeds from said plant, and (d) collecting the seeds so produced by said transformed plant.

67  
68.

The method of claim 67, said method further comprising the step of (e) transforming said plant cell with at least a second polynucleotide that encodes at least one enzyme involved in phosphatidylcholine biosynthesis, wherein said second polynucleotide is operably linked to a promoter capable of expressing said second polynucleotide in said plant cell to produce said enzyme in said cell.

68  
69.

A method for increasing the lipid content of a plant seed, said method comprising the steps of (a) growing the transgenic plant of claim 47, under conditions effective to produce seed in said plant, and (b) obtaining the seed produced from said plant.

69  
70.

The method of claim 69, wherein said seed are corn, rice, millet, tobacco, alfalfa, soybean, bean, sorghum, pea, *Brassica*, sugar beets, safflower, potato, tomato, flax, canola, sunflower, cotton, kapok, wheat, oat, barley, or rye seed.

70  
71.

A method for improving the osmotic stress tolerance of a plant, said method comprising the steps of (a) transforming said plant with a first selected nucleic acid segment that

comprises the polynucleotide of claim 1, and a second selected nucleic acid segment that comprises a polynucleotide encoding choline monooxygenase, choline oxidase, or choline dehydrogenase, each of said selected nucleic acid segments operably linked to a promoter that drives expression of said segments in said plant; and (b) growing the plant so transformed under conditions effective to improve the osmotic stress tolerance of said plant.

71  
72.

10 The method of claim 71, wherein the level of glycine betaine or choline-*O*-sulfate in said transformed plant is elevated relative to the level of glycine betaine or choline-*O*-sulfate in an untransformed plant of the same species.

72  
73.

15 The method of claim 72, further comprising the step of providing to the cells of said transformed plant ethanolamine or phosphoethanolamine in an amount effective to increase the level of phosphocholine in the cells of said plant.

73  
74.

20 The method of claim 72, wherein said providing comprises transforming said plant with a third selected nucleic acid segment that encodes an ethanolamine biosynthetic enzyme, wherein said segment is operably linked to a promoter that drives expression of said segment in said plant.

74  
75.

25 A method for increasing the cryoprotectant properties of a plant, said method comprising the steps of (a) transforming said plant with a first selected nucleic acid segment that comprises the polynucleotide of claim 1, and a second selected nucleic acid segment that comprises a polynucleotide encoding choline monooxygenase, choline oxidase, or choline dehydrogenase, each of said selected nucleic acid segments operably linked to a promoter that drives expression of said segments in said plant; and (b) growing the plant so transformed under conditions effective to improve the osmotic stress tolerance of said plant.

30

A recombinant vector designated pREP3-PEAMT or pREP3- $\Delta$ PEAMT.

The vector of claim 76, comprised within a host cell.

5

Ad AB

**THE** **NEW** **YORK** **PUBLIC** **LIBRARY**